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Differentiation of the Midbrain Dopaminergic Pathways during Mouse Development

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ABSTRACT

Dopaminergic (DA) neurons in the substantia nigra (SN) and ventral tegmental area (VTA) of the midbrain project to the dorsolateral caudate/putamen and to the ventromedially located nucleus accumbens, respectively, establishing the mesostriatal and the mesolimbic pathways. Disruptions in this system have been implicated in Parkinson's disease, drug addiction, schizophrenia, and attention deficit hyperactivity disorder. However, progress in our understanding has been hindered by a lack of knowledge of how these pathways develop. In this study, different retrograde tracers, placed into the dorsolateral caudate/putamen and the nucleus accumbens, were used to analyze the development of the dopaminergic pathways. In embryonic day 15 mouse embryos, both SN and VTA neurons, as well as their fibers, were doubly labeled by striatal injections into the dorsolateral and ventromedial striatum. However, by birth, the SN DA neurons were labeled exclusively by DiA placed in the dorsolateral striatum, and the VTA DA neurons were labeled only by DiI injected into the ventromedial striatum. These data suggest that initial projections from midbrain DA neurons target nonspecifically to both the dorsolateral striatum and the nucleus accumbens. Later during development, the separate mesostriatal and mesolimbic pathways differentiate through the selective elimination of mistargeted collaterals. J. Comp. Neurol. 476:301–311, 2004.

Indexing terms: axon tracing; substantia nigra; ventral tegmental area; striatum

Dopaminergic neurons in adult rodent midbrain are located primarily in two adjacent regions, the more lateral substantia nigra (SN; A8 and A9 groups of Dahlstrom and Fuxe, 1964) and the more medial ventral tegmental area (VTA; A10 group). In rats, these neurons begin to develop around E11 (Bayer et al., 1995), under the influence of sonic hedgehog and FGF signals (Arenas, 2002). Their axons first migrate dorsally and then turn abruptly toward the anterior (Nakamura et al., 2000). By E13, almost all dopaminergic axons have turned anteriorly, heading toward the forebrain targets. DA axons arrive at the striatal target around E15–E16 (Voorn et al., 1988).

In the mature rat brain, dopaminergic axons terminate topographically in the striatal, limbic, and cortical telencephalic regions (Simon et al., 1976, 1979; Carter and Fibiger, 1977; Fallon and Moore, 1978; Moore and Bloom, 1978; Nauta et al., 1978; Beckstead et al., 1979; van der Kooy, 1979; Lindvall and Bjorklund, 1983). DA neurons located in the SN send axons mainly to the dorsolateral striatum (dorsolateral caudate-putamen), forming the nigrostriatal pathway. DA neurons from the VTA, on the other hand, project mostly to the ventromedial striatum [which includes the medial caudate-putamen, the nucleus accumbens (NAc), and the olfactory tubercle; nomencla-

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ture according to Heimer et al. (1995)], constituting the mesolimbic pathway, and to the cortex (the mesocortical pathway).

It has been well established that the nigrostriatal pathway is essential for motor functions (Di Chiara et al., 1992); its degeneration is one of the major pathological changes in Parkinson's disease. In contrast, the mesolimbic pathway plays a key role in the motivational aspects of drug addiction as well as in emotion and goal-oriented behavior in general (Self and Nestler, 1995; Hyman, 1996). The correlation between the topographic distribution of dopaminergic projections to the striatum and the differentiation of the motivational and motor functions suggests that the topographic arrangement is critical to the functions of the midbrain dopaminergic system. However, the cellular and molecular mechanisms underlying dopaminergic pathway differentiation are not known. The current study aims at elucidating the cellular mechanisms of pathway differentiation of the midbrain dopaminergic neurons during embryonic development. This analysis examines the dynamics of dopaminergic axon targeting and shows that, when the axons first arrive at the striatum, the terminals are not topographically specific and that the specificity is achieved only later during development.

MATERIALS AND METHODS

Animals

Embryonic or postnatal CD1 mice of different developmental stages (E15, E17, P0, P7, P12, and adult) were used in this study. Adult (3–6-month-old) and timed-pregnant female mice were purchased from Charles River, Inc. (Wilmington, MA). At least three animals from each developmental stage were examined. The procedures for animal maintanance and experimentation used in this study were approved by the Institutional Animal Care and Use Committee.

Retrograde labeling of the midbrain dopaminergic neurons in postnatal mice

To investigate mesostriatal and mesolimbic topographic projections of the midbrain dopaminergic system in postnatal mice (P12 and adult), the retrograde tracer Fluoro-Gold (FG) was injected in the dorsolateral or ventromedial regions of the striatum. The animals were anesthetized, and a single iontophoretic injection of a 4% solution of FG in 0.9% NaCl was made through a stereotaxically positioned glass micropipette (tip diameter 10 μm) by applying a +0.5-μA current pulsed at 7-second intervals, provided by an alternating-current source (Midgard TM Electronics) for a period of 20 minutes. This condition generated an injection site of approximately 10–15% of the striatum. The animals were allowed to survive for 7 days after injection, then perfused with phosphate-buffered saline (PBS; pH 7.4) containing heparin sodium (10 U/100 ml), followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4). The specimens were kept overnight in 0.1 M PBS containing 30% sucrose at 4°C. Subsequently, 50-μm-thick coronal sections were made with a freezing microtome and observed under a fluorescence microscope.

Retrograde labeling of the midbrain dopaminergic neurons in embryonic or early postnatal mice

Dopaminergic neurons in embryonic and early postnatal mice were retrogradely labeled using DiA and DiI double injections. Mouse embryos were dissected, decapitated, and immediately immersed in 4% paraformaldehyde in 0.5 M PBS for 1 week at 4°C before dye injection. Postnatal pups (P0–P7) were cryoanesthetized with ice, then perfused as described above. The fixed brains were dissected, and the frontal portion was removed to expose the striatal areas. The brains were then positioned such that the cut surface was facing upward, in 1% agarose in PBS. Approximately 0.1 μl of a 10% DiA solution in N,N-dimethyl-formamide (DMF) was injected into the dorsolateral striatum (caudate/putamen), and 0.1 μl of 10% DiI in DMF was placed in the nucleus accumbens of the fixed mouse brains under a surgical microscope, using a fine capillary glass pipette (see Fig. 3). Injected brains were then examined under a dissection microscope equipped with epifluorescence, and any brains with bad injections in which DiI and DiA injection sites overlapped were discarded. Properly injected brains were then incubated for 2 months in 4% paraformaldehyde containing 30% sucrose at 37°C, and 100-μm horizontal sections were cut with a vibrotome, observed with a Zeiss Axiovert 100M confocal microscope, and recorded with a digital camera connected to the microscope (AxioCam MRm; Zeiss).

Tyrosine hydroxylase immunohistochemistry

For tyrosine hydroxylase (TH) immunostaining, free-floating sections prepared as described above were incubated in anti-TH rabbit antisera (polyclonal antibody, 1:250; Chemicon, Temecula, CA), followed by incubation with biotinylated goat anti-rabbit IgG for 4 hours (1:500; Vector Laboratories, Burlingame, CA) and with Cy3-conjugated egg-white avidin for 1 hr (1:500; Jackson Immunoresearch Laboratories, West Grove, PA).

Photomicrograph producton

Digital photographic images were combined into multipanel figures using Adobe Photoshop. No alterations were made other than that the contrast and brightness were adjusted accasionally to match different panels. No adjustments were made when the images with equal exposure times were used for quantification, to ensure accurate measurements.

RESULTS

Ascending midbrain dopaminergic pathways in adult mice

Earlier studies indicated that, in the adult rat, DA axons from the SN make connections with the dorsolateral striatum, and DA axons from the VTA target the ventromedial striatum (Simon et al., 1976, 1979; Carter and Fibiger, 1977; Fallon and Moore, 1978; Moore and Bloom, 1978; Nauta et al., 1978; Beckstead et al., 1979; van der Kooy, 1979; Lindvall and Bjorklund, 1983). To examine whether these distinct pathways are similarly preserved in the mouse, we first retrogradely labeled the adult mid-

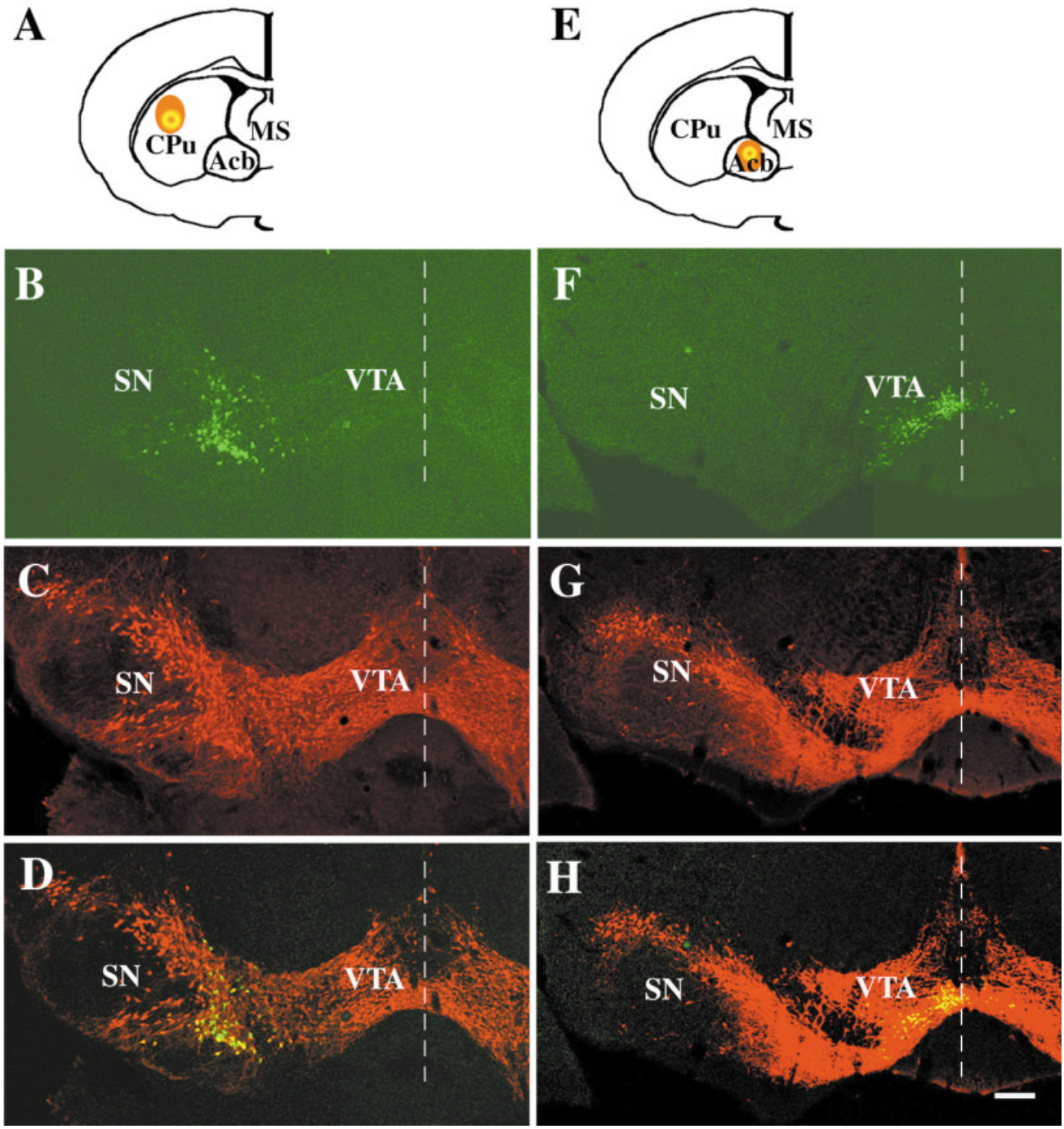

Fig. 1. Topographic projections in the midbrain dopaminergic system in adult mice. **A,E:** Diagrams indicating Fluoro-Gold injection sites in the dorsolateral or ventromedial striatum. **B,F:** SN and VTA neurons retrogradely labeled with Fluoro-Gold (greenish gold), respectively. **C,G:** The traced brain sections stained with anti-TH antibody (red). **D,H:** Overlapping images of Fluoro-Gold- and TH-labeled midbrain cells showing distinct projection targets for different DA neurons (yellow) in mesostriatal (left panels) or mesolimbic (right panels) pathway. Dashed lines indicate the midline. Acb, nucleus accumbens; Cpu, caudate/putamen; MS, medial septum; SN, substantia nigra; VTA, ventral tegmental area. Scale bar = 100 μm.

brain dopaminergic neurons by placing the retrograde tracer FG in either the dorsolateral or the ventromedial striatum of the adult mice. The traced midbrains were sectioned and then immunostained with anti-TH antibody coupled with Cy3-conjugated secondary antibody to identify the position of dopaminergic neurons. When FG was injected in the dorsolateral striatum, only dopaminergic neurons located in the SN were labeled (Fig. 1A–D). In

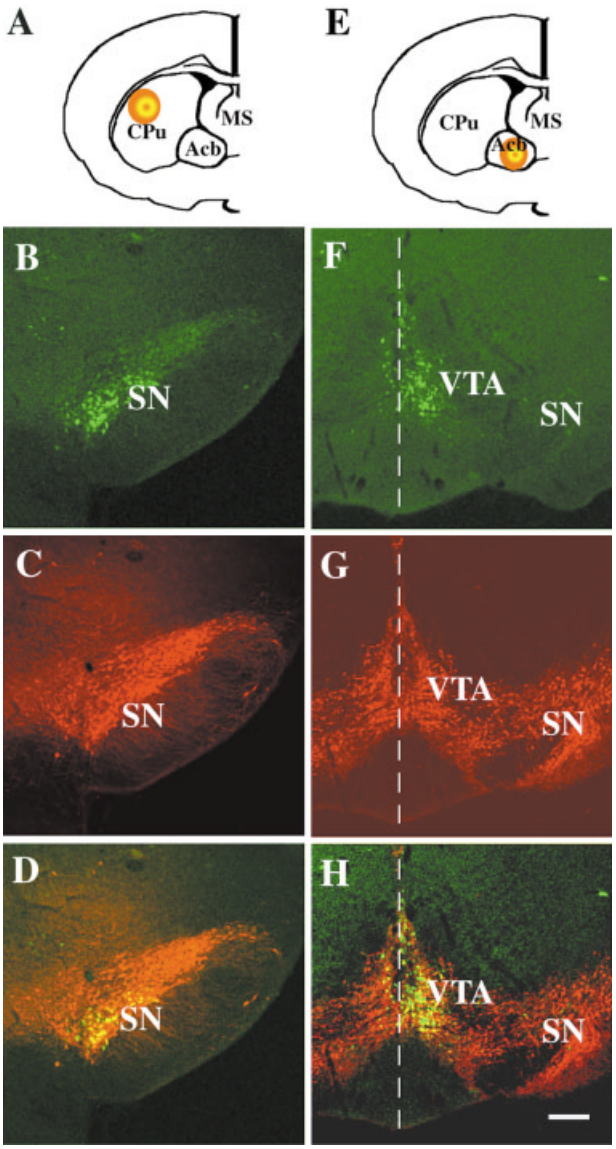

Fig. 2. Topographic projections in midbrain dopaminergic system in P12 mice. **A,E:** Diagrams showing Fluoro-Gold injection sites in the dorsolateral (A) and ventromedial (E) striatum. **B,F:** SN and VTA neurons retrogradely labeled with Fluoro-Gold, respectively (green). **C,G:** The Fluoro-Gold-traced sections stained with anti-TH antibody (red). **D,H:** Overlapping images of Fluoro-Gold- and TH-labeled midbrain neurons showing distinct projection targets for different DA neurons (yellow) in mesostriatal (left panels) or mesolimbic (right panels) pathway. Dashed lines in F–H indicate the midline. For abbreviations see legend to Figure 1. Scale bar = 100 μm.

contrast, when the retrograde tracer was injected in the ventromedial striatum, only dopaminergic neurons located in the VTA were labeled (Fig. 1E–H). Similar results were obtained when P12 mouse brains were analyzed (Fig. 2). These analyses indicate that, in the mouse, as in the rat, there are clearly differentiated mesostriatal and mesolimbic pathways. Furthermore, these pathways are well established by P12, suggesting that any developmental program underlying the specification of these distinct midbrain dopaminergic pathways must operate before P12.

Lack of pathway specificity in the early midbrain dopaminergic projections

The midbrain dopaminergic neurons begin to separate into the SN and the VTA around E16 in the rat (equivalent to E14–E15 in the mouse). Therefore, to examine whether dopaminergic axons from the SN and VTA form specific pathways when they first arrive at the striatum, E15 embryonic mouse brains were traced as described in Materials and Methods, with DiA injected in the dorsolateral striatum and DiI in the ventromedial striatum (Fig. 3). Because the embryonic brains were relatively small, great care was taken to avoid dye spread. In addition, each injected brain was examined under a dissection microscope equipped with epifluorescence, and brains with overlapping injection sites were discarded. Only brains with well-separated injection sites were used for analyses. Quantitative measurements showed that average sizes of injection sites for E15 brains were 5–5.6% of the striatum; for P0 brains, the sizes were 4.8–7% of the striatum. The DiA and DiI injection sites were well separated, 0.62 mm on average for E15 brains and 0.72 mm for P0 brains (Fig. 3). To examine the patterns of retrogradely labeled midbrain neurons, injected brains were sectioned horizontally to allow examination of both the midbrain neurons and the axon pathways to the striatal targets. In E15 embryos, the midbrain neurons located in both the SN and the VTA were colabeled by DiA and DiI (Fig. 4A–C). Cell soma with both red and green labels were clearly identifiable along with the proximal axons (Fig. 4D–F and G–I). In addition, axons on their way to the striatum were also doubly labeled by DiA and DiI (Fig. 4J–L). These observations indicate that the midbrain axon collaterals target both the dorsolateral and the ventromedial striatum in E15 mouse embryos.

Dopaminergic pathways differentiate during late embryogenesis

At E17, significant numbers of the midbrain neurons are still doubly labeled by DiA and DiI (Fig. 5A–F), indicating that there are still large numbers of axon collaterals projecting to both the dorsolateral and the ventromedial striatum. However, more single-labeled neurons begin to appear (Fig. 5A–C). In addition, the axons on the way to the dorsolateral and ventromedial striatum begin to segregate (Fig. 5G–I), with only some double-labeled axons. These observations indicate that the mesostriatal and the mesolimbic pathways have begun to differentiate by E17 in the mouse.

Establishment of the mouse midbrain dopaminergic pathway specificity by birth

DiA and DiI double retrograde tracing analysis showed that the specificity of the ascending midbrain dopaminergic pathways has been established by the time of birth (P0; Fig. 6). In newborn mice, DiA injected into the dorsolateral striatum labeled the SN neurons, and DiI injected into the ventromedial striatum traced the VTA neurons. No double-labeled neurons were observed at P0, indicating that the SN to the dorsolateral striatum (mesostriatal) and the VTA to the ventromedial striatum (mesolimbic) pathways had been differentiated by this time. Consistent with a clear differentiation of the mesostriatal and the mesolimbic pathways, no axon fibers on their way to the striatum were found to be doubly labeled, although there

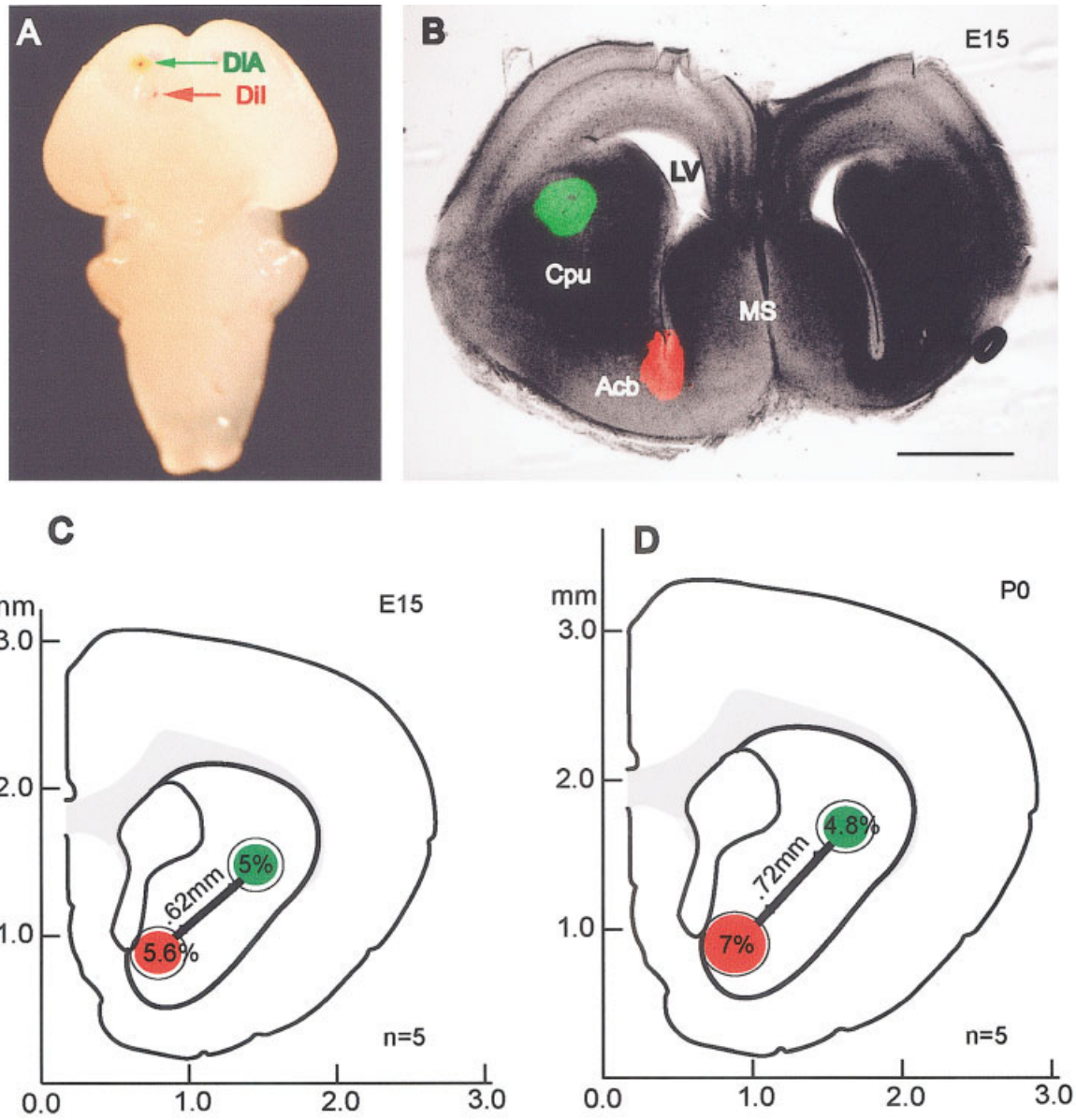

Fig. 3. DiA and DiI applications in embryonic and postnatal mice. In this and subsequent analyses, DiA was always applied in the dorsolateral striatum and DiI always in the ventromeidal striatum. **A:** Ventral view of a fixed E15 embryo brain labeled with DiA and DiI. The embryonic brain was positioned such that the cut surface could be easily visualized. **B:** Coronal section of a labeled brain through the injection sites. Green is DiA, and red is DiI. Note that the injection sites are well separated. **C,D:** Diagrams showing the percentage of striatum labeled with DiI (red) or DiA (green) and the average distance between the two dyes in the analyses of E15 (C) or P0 (D) brains. The circles outside the colored area indicate standard deviation. The scales in C and D are in millimeters. Acb, nucleus accumbens; CPu, caudate/putamen; LV, lateral ventricle; MS, medial septal nucleus. Scale bar = 1 mm.

was some spatial overlap of the mesostriatal and mesolimbic axons that were labeled, respectively, by DiA and DiI (Fig. 6E–H). At P7, the specificity of the ascending midbrain dopaminergic pathways was similar to that of the later stages of mice (Fig. 7A–D).

A quantitative analysis indicated that the specificity of the ascending midbrain dopaminergic pathways was established between E15 and P0 (Fig. 7E,F, Table 1). At E15, a large percentage of the midbrain neurons (62.6%) was doubly labeled. By E17, the fraction of double-labeled neurons had decreased sharply (13.1%). By P0, no double-labeled neurons were observed.

DISCUSSION

The midbrain dopaminergic pathways play central roles in motor controls and brain reward responses and are involved in neurological disorders such as Parkinson's disease and drug addiction. In the treatment of Parkinson's disease, transplantation of dopaminergic neurons is

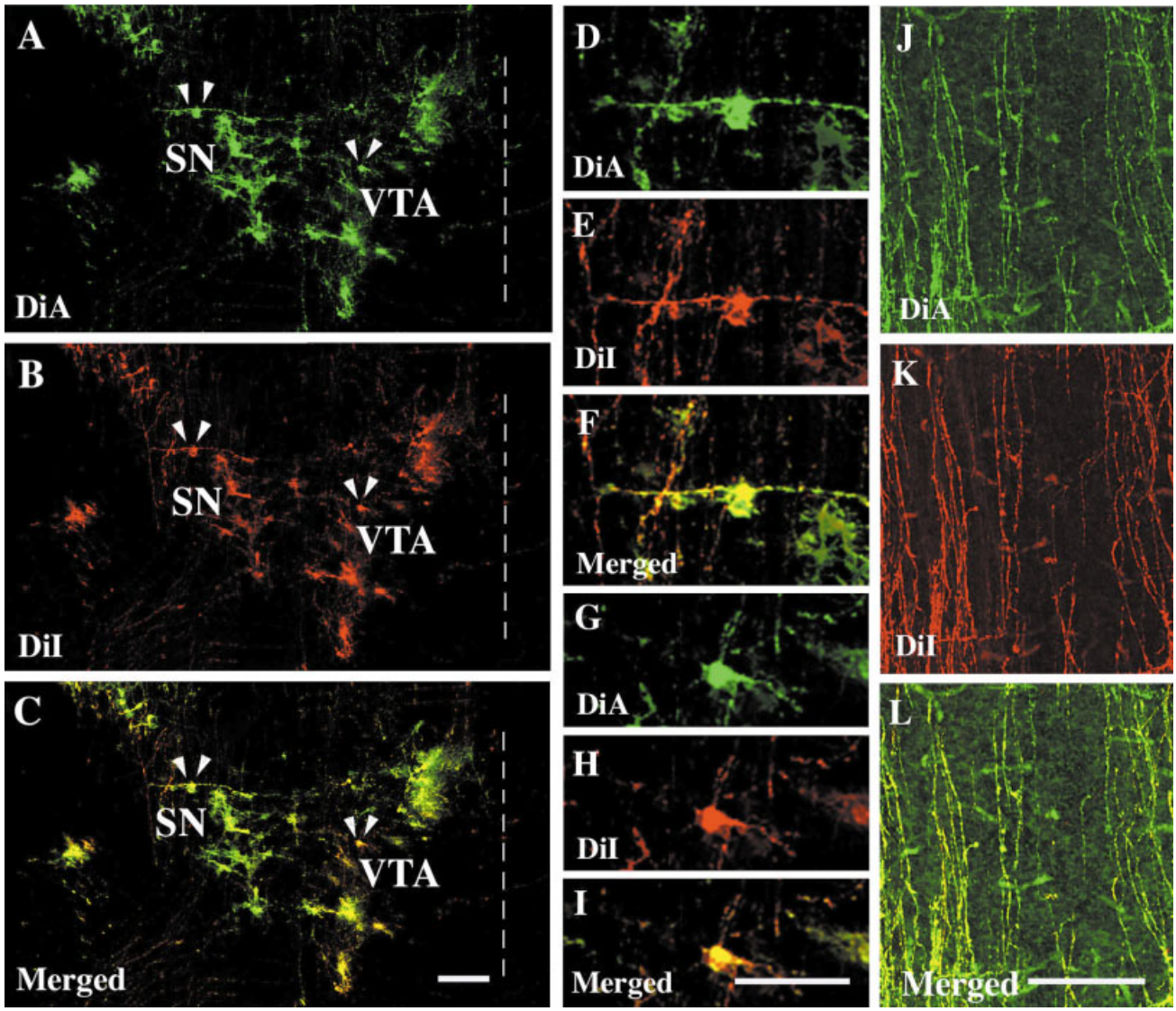

Fig. 4. The midbrain to striatum projection exhibited no specificity in E15 mice. **A,D,G,J:** Midbrain neurons and their axon fibers retrogradely labeled with DiA. **B,E,H,K:** Midbrain neurons and their axon fibers retrogradely labeled with DiI. **C,F,I,L:** Merged images of corresponding DiA and DiI-labeled midbrain neurons. Arrowheads in A–C indicate colocalization of DiA and DiI-labeled neurons, which are shown in D–I at higher magnification. Dashed lines in A–C indicate the midline. SN, substantia nigra; VTA, ventral tegmental area. Scale bars = 100 μm in C (applies to A–C); 100 μm in I (applies to D–I); 100 μm in L (applies to J–L).

an attractive alternative to the current drug therapy (Freed et al., 2001). Although DA neurons can now be generated in vitro and potentially used in cell-replacement therapy (Arenas, 2002; Kim et al., 2002), little is known about how functional DA pathways can be reconstructed (Ma et al., 2002). The current study shows that, during development, the midbrain dopaminergic axons from the SN and the VTA initially project nonspecifically to both the dorsolateral and the ventromedial striatum. The specificity of the axon pathways develops in late embryogenesis, and, by P0, dopaminergic axons from the SN target only the dorsolateral striatum, and the axons from the VTA project mainly to the ventromedial striatum.

Methodological considerations of retrograde axon tracing in embryonic mouse brain

Because the embryonic brains are rather small, we were concerned that the DiA and DiI injected might cross-contaminate, resulting in double labeling of midbrain DA neurons. Several precautions were taken to prevent this potential problem. First, a very small volume, about 0.1 μl, of dye was injected. Second, injected brains were examined first under a dissection microscope, and any brains with dye cross-contamination were excluded from the analyses. Finally, a detailed analysis of the injection sites and the distance between DiA and DiI injection sites was performed and showed that the injections were highly

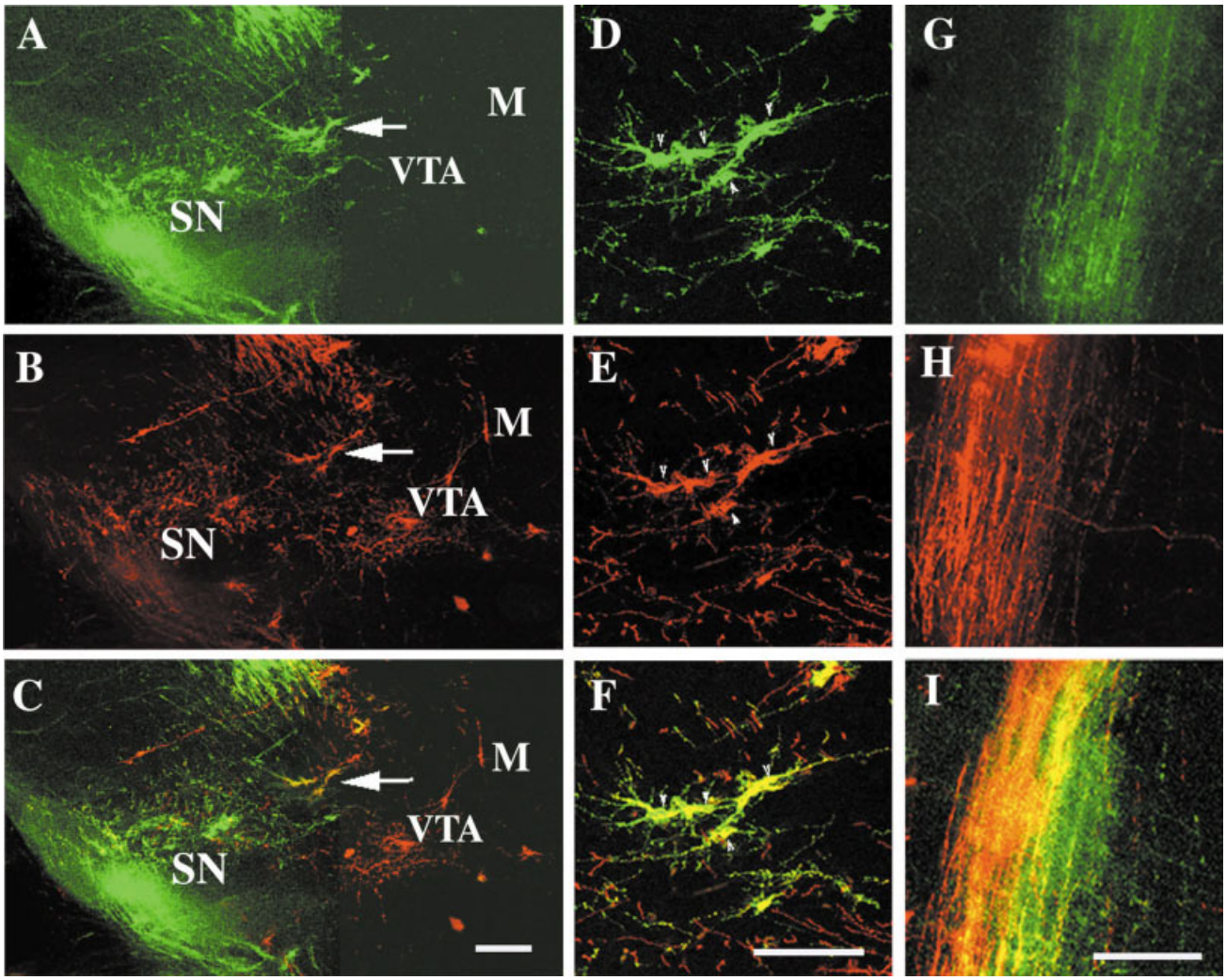

Fig. 5. Emerging specificity in midbrain dopaminergic system in E17 mice. **A,D,G:** Midbrain neurons and axons retrogradely labeled with DiA. **B,E,H:** Midbrain neurons and axons retrogradely labeled with DiI. **C,F,I:** Merged images of DiA- and DiI-labeled midbrain neurons and axons. Dashed lines in A–C indicate the midline. Arrows in A–C indicate DiA/DiI double-labeled neurons that are shown at higher magnification in D–F. SN, substantia nigra; VTA, ventral tegmental area. Scale bars = 100 μm in C (applies to A–C); 100 μm in F (applies to D–F); 100 μm in I (applies to G–I).

restricted and that there was no cross-contamination of the injected dyes. Our studies indicated that, at E15, most of the midbrain neurons were doubly labeled. However, by P0, no midbrain neurons were doubly labeled (Fig. 7E,F). The P0 striatum is only about 30% larger than the E15 striatum (Jacobowitz and Abbott, 1997). The size difference, therefore, could not explain the dramatic difference in the percentage of double-labeled midbrain neurons (62.6% in E15 vs. 0% in P0; Fig. 7E,F). In addition, the brains analyzed all had proper injections in which the two dyes did not overlap. Thus, the double labeling of the midbrain neurons should reflect the presence of collaterals projecting to distinct regions of the striatum.

Development of specificity of the midbrain dopaminergic pathways

The midbrain axons targeting the striatum initially project to both the dorsolateral and the ventromedial striatum; both the axons and the cell soma are doubly labeled by DiA injected in the dorsomedial striatum and DiI injected in the ventromedial striatum. Dopaminergic axons are known to have numerous collaterals targeting a large number of target neurons (Lindvall and Bjorklund, 1979; Fallon and Loughlin, 1982; Ikai et al., 1994). During early embryogenesis, axon collaterals derived from each neuron may target nonspecifically to different regions of the striatum, which is consistent with the observation that many of the midbrain neurons are doubly labeled with retrograde axon tracers injected into different locations in the striatum. There is a gradual shift for the midbrain neurons from being mostly doubly labeled to entirely singly labeled from E15 to P0, indicating that, by the time of birth, the distinct midbrain dopaminergic pathways have been fully differentiated. There are several potential mechanisms by which this differentiation may be achieved. First, double-labeled neurons, along with their

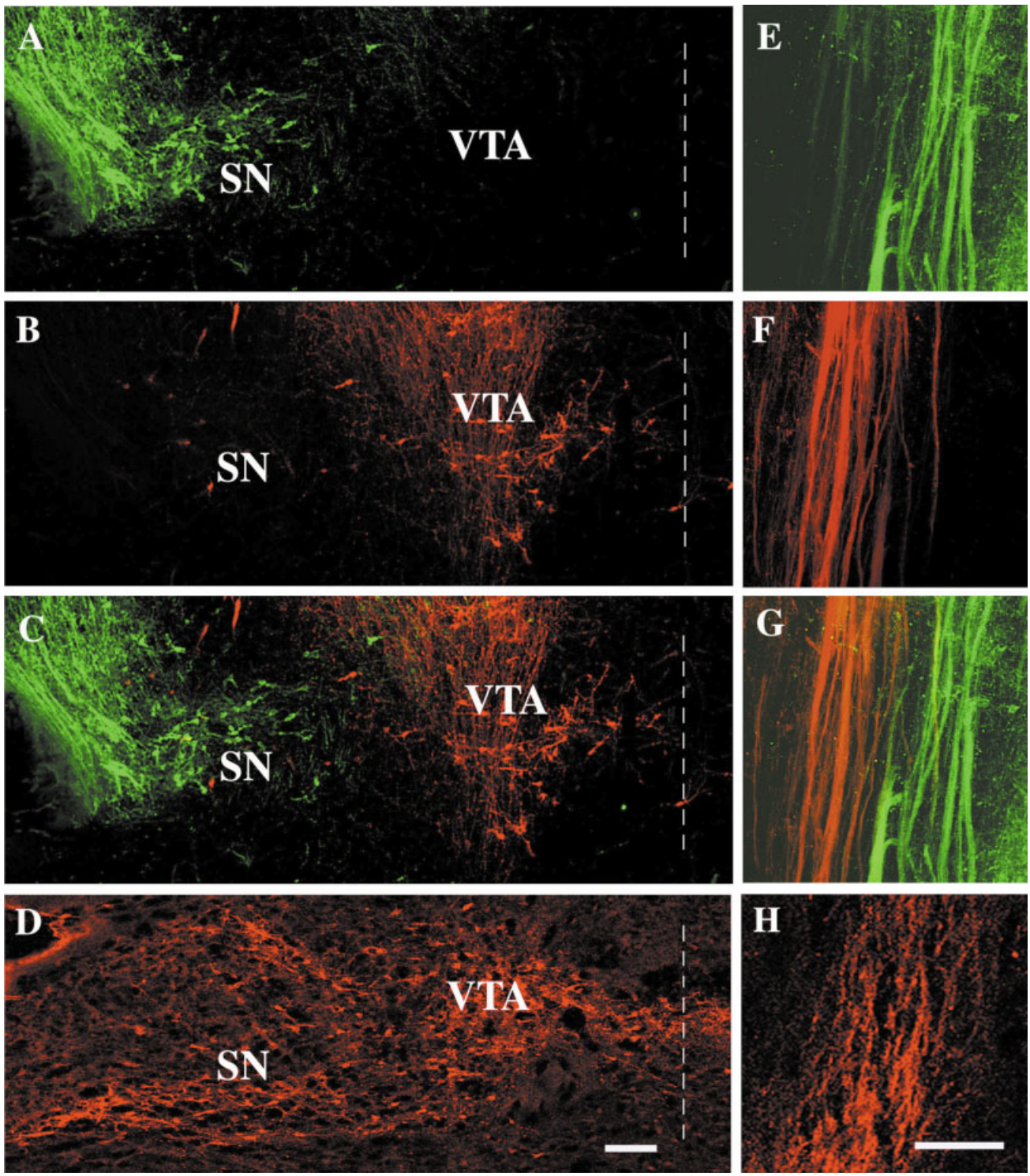

Fig. 6. The midbrain to striatum DA projections are topographically specific in P0 mice. **A,E:** Midbrain neurons and axons retrogradely labeled with DiA. **B,F:** Midbrain neurons and axons retrogradely labeled with DiI. **C,G:** Merged images of DiA and DiI-labeled midbrain neurons and axons. **D,H:** Same regions of the brain on serial sections were stained with anti-TH to indicate positions of the dopaminergic neurons and axons. Dashed lines in A–D indicate the midline. SN, substantia nigra; VTA, ventral tegmental area. Scale bars = 100 μm in D (applies to A–D); 100 μm in H (applies to E–H).

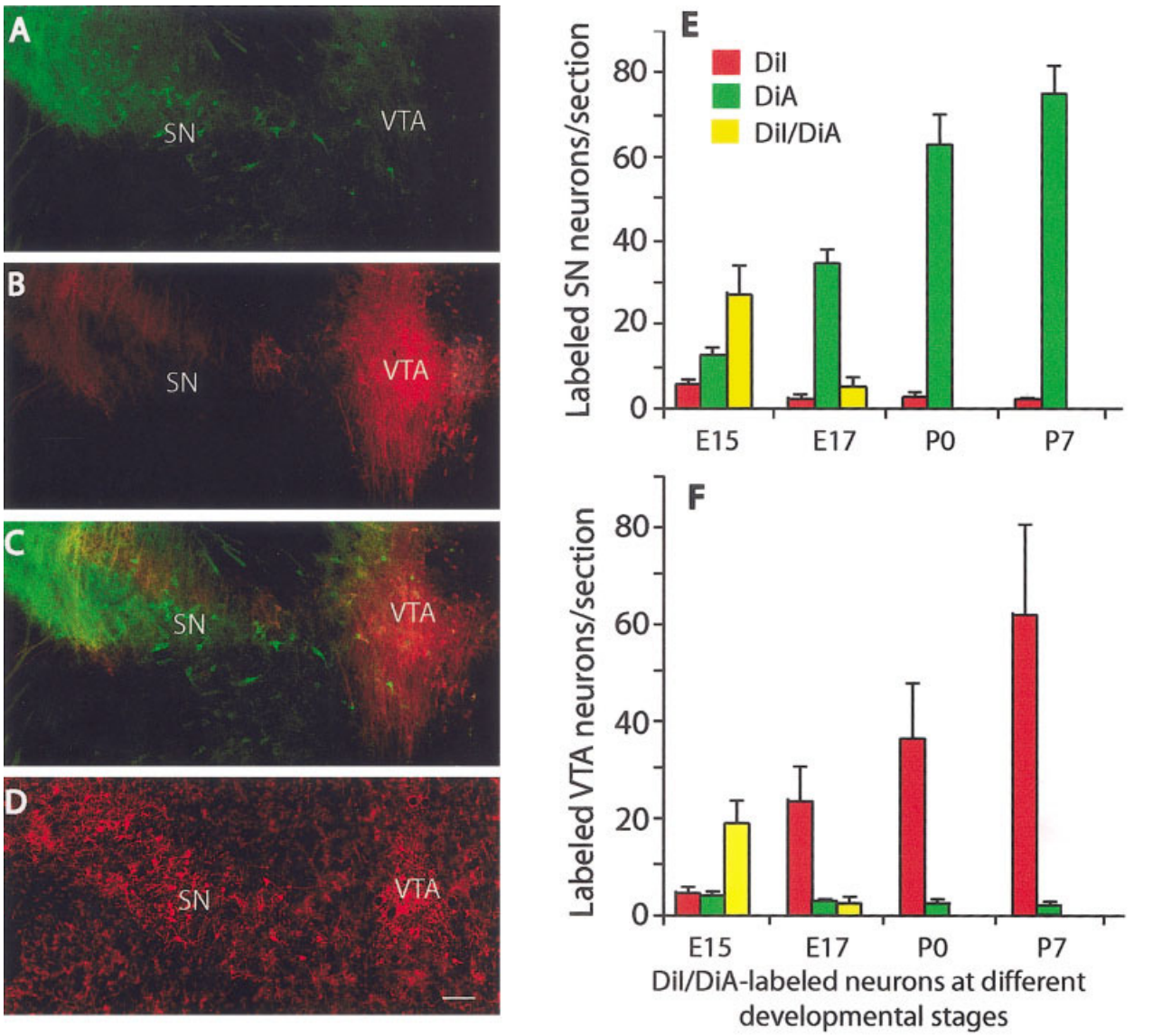

Fig. 7. A–D: Topographic projections in the midbrain dopaminergic system in P7 mice. **A:** Midbrain neurons retrogradely labeled with DiA. **B:** Midbrain neurons retrogradely labeled with DiI. **C:** Merged image of DiA- and DiI-labeled ventral midbrain neurons. **D:** A neighboring section stained with anti-TH immunocytochemistry to indicate the position of the dopaminergic neurons. **E,F:** Quantification of double-labeled midbrain neurons at different developmental stages. The proportions of DiA/DiI double-labeled neurons were shown as the mean ± SEM. Quantification was made in two typical sections that contain most of the traced neurons in both SN and VTA from each animal analyzed, and data from six individual animals were collected for each group. SN, substantia nigra; VTA, ventral tegmental area. Scale bar = 100 μm.

axons, may be eliminated by developmentally regulated cell death. It has been shown that significant numbers of neurons die during embryonic development (Oppenheim, 1991). The number of neurons and axons that survive is determined by the availability of appropriate targets, which may provide trophic factors to sustain the neurons through retrograde mechanisms (Yuen et al., 1996). This target-derived neurotrophic mechanism has been proposed to play an important role in the establishment of appropriate neural pathways (Barde, 1989; Clarke et al., 1998). However, this mechanism would require a rather extensive removal of differentiated dopaminergic cells, because most of the cells have mistargeting collaterals. In addition, there is no evidence at present that extensive cell death occurs during development of the midbrain dopaminergic pathways before birth (Oo and Burke, 1997; Jackson-Lewis et al., 2000; Oo et al., 2003). Thus, although this mechanism is formally possible, we consider it not very likely. Second, migration of the differentiated dopaminergic neurons to segregate into the SN and the VTA may also play a role in the formation of the distinct pathways. However, the migration of the cell soma alone cannot lead to the loss of the mistargeting collaterals. Third, mistargeting collaterals may be pruned during late

TABLE 1. Number of Midbrain Neurons Labeled by Retrograde Axon Tracers[1]

Labeling	E15	E17	PO	P7
SN				
Dil	4.83 ± 2.04	3.00 ± 0.89	3.67 ± 0.82	3.00 ± 0.89
DiA	12.80 ± 1.75	34.50 ± 1.97	62.83 ± 3.71	77.17 ± 2.45
Dil/DiA	27.67 ± 1.50	5.67 ± 2.32	0	0
Total	44.17 ± 2.64	43.17 ± 2.32	66.83 ± 3.60	78.33 ± 3.20
VTA				
Dil	4.50 ± 0.96	23.00 ± 1.91	35.83 ± 1.47	61.17 ± 3.92
DiA	3.83 ± 1.47	2.33 ± 0.52	2.17 ± 0.41	2.50 ± 0.84
Dil/DiA	19.00 ± 1.79	2.33 ± 0.52	0	0.83 ± 1.00
Total	27.33 ± 2.58	27.67 ± 2.88	38.00 ± 1.53	63.67 ± 4.50

[1]Data collected from a total of six successfully traced animals for each time point.

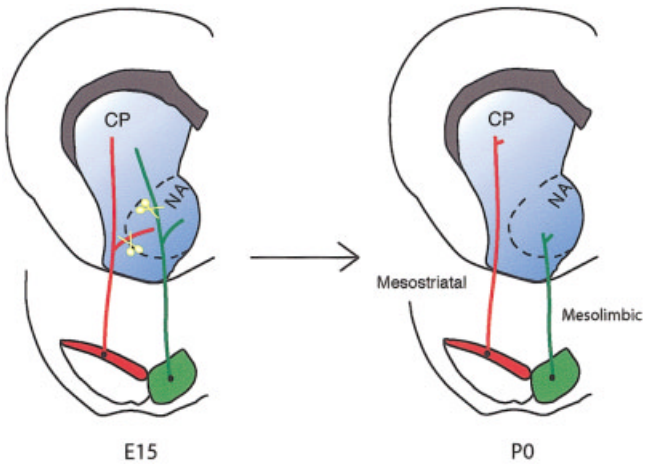

Fig. 8. A model of development of the midbrain dopaminergic pathways. In the initial phase of development of the midbrain dopaminergic pathways, axon collaterals target nonspecifically to the striatum. Pathway differentiation occurs by specific removal of mistargeting collaterals later in development. Blue color in the striatum indicates expression of ephrin-B2, and red color represents EphB1 expression. Green indicates potential expression of uncharacterized axon guidance cues in the VTA DA neurons and axons. Molecular interactions between ephrin-B2 and EphB1 as well as regulations by other axon guidance signals may be important for DA axon pathway differentiation.

embryogenesis by the presence of pruning signals in the inappropriate target areas. Axon pruning has been shown to regulate the formation of many neural pathways, including the visual topographic map in the tectum (Simon and O'Leary, 1990, 1992; Bagri et al., 2003; Watts et al., 2003). Consequently, axon pruning may play a key role in the establishment of specific axon pathways and in the establishment of topographic maps. The distinct midbrain dopaminergic pathways could be similarly specified by pruning of mistargeted collaterals (Fig. 8).

Axon guidance and pruning signals for the midbrain dopaminergic axons

The nature of axon guidance and pruning signals regulating DA pathway specificity is not known at present. There is evidence that the spatial positions of mature DA axon terminals may be determined by the spatially specific patterns of ephrin and Eph receptor expression (Yue et al., 1999). It has been shown that EphB1, a receptor of the Eph family, is expressed at high levels in the SN but at low levels in the VTA (Yue et al., 1999). Complementary to EphB1 expression, ephrin-B2, a ligand of EphB1 (Brambilla et al., 1995), is transcribed at high levels in the ventromedial region of the striatum, including the nucleus accumbens and olfactory tubercle, but at low levels in the dorsolateral striatum. Thus, SN dopaminergic neurons expressing high levels of EphB1 receptor project to the dorsolateral striatum, which expresses low levels of the ligand ephrin-B2 (Fig. 8). In contrast, VTA neurons, which express low levels of the receptor, project to the ventromedial striatum, where high levels of ephrin-B2 are transcribed (Fig. 8). The complementary expression of Eph receptor and ligand in presynaptic and postsynaptic fields suggests an inhibitory interaction to restrict SN DA axons in dorsolateral striatal targets. Indeed, in vitro studies revealed that ephrin-B2 inhibited growth of EphB1-positive SN DA axons (Yue et al., 1999). Although a pruning activity has not been demonstrated for DA axons, ephrin-A ligands have been shown to result in axon degeneration of hippocampal neurons (Gao et al., 1999). These observations suggest that the Eph-family receptors and ligands may interact to restrict SN collaterals to the dorsolateral striatum and may function as pruning factors to remove the mistargeting collaterals (Fig. 8).

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